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Final Report

Development of an Integrated Approach to The Control of Bacterial Diseases of Tomato And Pepper

REPORT No. OREP - 1999/08

LEAD INVESTIGATOR

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Canada



**ONTARIO RESEARCH ENHANCEMENT PROGRAM
(O R E P)**

**DEVELOPMENT OF AN INTEGRATED APPROACH
TO THE CONTROL OF BACTERIAL DISEASES OF
TOMATO AND PEPPER**

**REPORT No. OREP-1999/08
July 2001**

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FORWARD

The Ontario Research Enhancement Program (OREP) is a \$4-million two-year federal research initiative that is administered by the Research Branch of Agriculture and Agri-Food Canada (AAFC), with input from the agriculture and agri-food sector, universities and the province. Research focuses on two areas identified by the sector as:

1. Priorities responding to consumer demand for higher quality products; and
2. Ensuring crop-production management systems are environmentally sustainable.

Biotechnology is one of the areas to be explored. Ontario's agricultural production is based primarily on growing diversified crops using intensive production practices, but long-term viability is linked to the development of sustainable crop production management systems.

There are also opportunities to enhance the economic contribution of Ontario's agriculture and agri-food sector by adding value to the diversified primary commodities produced in the province. The Program is expected to be of particular interest to the corn, soybean, greenhouse, fruit and vegetable sectors and the emphasis will be on projects focussing on food quality improvement and sustainable crop production management such as:

1. The development of value-added animal and crop food products and ingredients that meet domestic and export market demands, with a particular emphasis on quality issues such as processing, packaging, shelf life, new product and ingredient development, high-value alternative uses, probiotics and functional foods;
2. Biotechnologies to reduce environmental pressures related to crop protection practices; and,
3. Integrated crop protection and production practices for both field and greenhouse crops.

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EXECUTIVE SUMMARY

The estimated farm value of the Ontario field tomato and pepper crop is approximately 100 million dollars a year. When one also considers the seed producers and food processing industry, these crops are vital to the economic health of southwestern Ontario. Over the last few years bacterial spot has been listed as one of the worst pest problems to face the tomato and pepper grower. Bacterial speck, often found in association with bacterial spot on tomato plants, and bacterial canker put additional stress on the plants. In a bad year canker can cause devastating losses. Because there are no tomato and pepper cultivars resistant to all races of bacterial spot or speck or to bacterial canker, the grower must rely on copper-based bactericides for control of these diseases. The recommended frequency for these sprays is every 5 to 7 days. Unfortunately, copper resistance is on the increase in Ontario populations of all three pathogens and eventually may preclude the use of these bactericides. The objective of the present study is to develop a method of controlling bacterial diseases of tomatoes and peppers that is based on the integrated use of microbial antagonists and biorational chemicals that induce disease resistance in plants. The specific goals are: (1) to determine, in growth room trials, the disease (bacterial and fungal) control efficacy of treating transplant seedlings with a microbial or chemical inducer of systemic acquired resistance (SAR), (2) to determine the control efficacy of these treatments on field plants, 3) to determine the effect of these agents on plant growth and yield and 4) to determine the control efficacy of combining chemical SAR inducers with microbial biocontrol agents. Results indicated that the SAR inducer benzothiodiazole (BTH), particularly when used in combination with selected bacterial biocontrol agents, was as effective, if not more so, than Kocide-Bravo mixtures in controlling bacterial diseases on field tomato and pepper. No detrimental effect on fruit yield was

observed. Particularly significant was the control of bacterial spot of field pepper, where disease severity was reduced an average of 60% during a 3-year field trial. Furthermore, BTH shows promise as a means of controlling the leaf blight fungal pathogen *Phytophthora infestans*. Such an effective disease management system will provide Ontario's tomato and pepper growers with an environmentally-friendly alternative to copper and thus should help to maintain the viability and competitiveness of the industry.



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INTRODUCTION

In 1998 the gross farm value of the Ontario field tomato and pepper crop was an estimated 78 million dollars (1998 Agricultural Statistics for Ontario) with over 20,000 acres being harvested. Over the last few years, the disease bacterial spot (*Xanthomonas campestris* pv. *vesicatoria*) (Fig. 1) has been a serious and persistent problem for both the processing and fresh market tomato and pepper grower, especially in northern Kent county. When disease conditions are optimal, infected plants lose blossoms, defoliate and produce a severely-spotted unsalable fruit. Although disease incidence and severity vary significantly from year to year, bacterial spot has reduced yield in some Kent county tomato fields by as much as 50% (Elmhirst, 1991). In 1990 and 1992 it caused an average loss of approximately 2 tons (~\$215)/acre and a 25-30% drop in fruit quality (J. Dick, Nabisco, personal communication). Similar yield losses have been reported in the U.S. (Kousik and Ritchie, 1994). Bacterial speck (*Pseudomonas syringae* pv. *tomato*), often found in association with bacterial spot in Ontario tomato fields, puts additional stress on the plants and contributes to yield loss. Because of the endemic nature of bacterial speck, it is difficult to obtain an accurate assessment of yield loss due to this disease. Bacterial canker (*Clavibacter michiganense* subsp. *michiganense*), a potentially devastating wilt disease of tomatoes, also is present in Ontario fields but disease incidence and plant loss vary from year to year. At present, there are no bacterial spot or bacterial canker-resistant tomato cultivars available to Ontario growers (J. Dick, Nabisco Brands, personal communication). Although there are bacterial speck-resistant and bacterial speck-tolerant cultivars grown in Ontario, strains of the pathogen that are able to overcome this resistance have been found by Lawton and MacNeill (1986). Thus control of

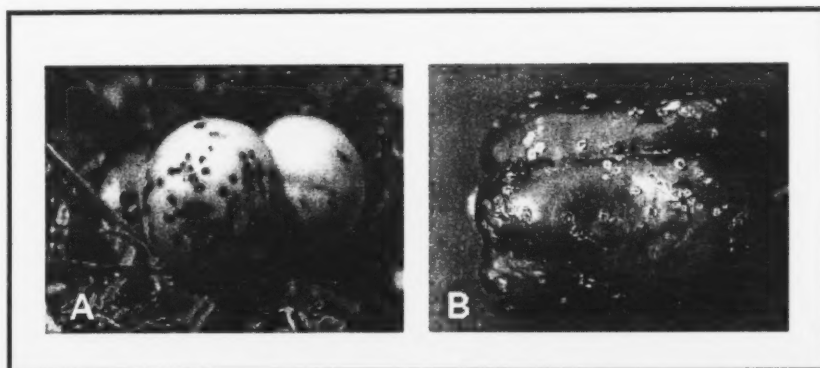


Figure 1. Bacterial spot lesions on field tomato (A) and pepper (B) fruits.

bacterial diseases of tomato and pepper in Ontario relies upon a vigorous spray program with copper-based bactericides. These bactericides are not foolproof; spray intervals of greater than 5-7 days or periods of heavy or frequent rainfall drastically reduce their effectiveness. **Unfortunately,** heavy use of copper eventually leads to widespread resistance to this chemical not only among strains of the pathogen but also among the other bacteria present in tomato and pepper fields (Cooksey, 1990; Marco and Stall, 1983). **High levels of copper resistance already have been detected in Ontario isolates of bacterial spot and bacterial speck** (Cuppels, unpublished data).

Over the last few years a large number of studies have focussed on the possibility of using introduced microbial antagonists to control plant disease (for general reviews see Cook, 1993; Cook et al., 1996; Handelsman and Stabb, 1996; Sivan and Chet, 1992; Wilson and Wisniewski, 1989; Weller, 1988). Microbial biocontrol agents (MBCA) hold enormous potential as a means of reducing our reliance on chemical pesticides while still maintaining crop yields (Cook et al., 1995). Relatively few of these studies have centered on the foliar diseases caused by bacteria (Windels and Lindow, 1985; Wilson and Lindow, 1993; Wilson and Lindow, 1994). Two previous studies, one involving the co-inoculation of greenhouse-grown tomato plants with *Pseudomonas syringae* pv. *tomato* and a copper-resistant nonpathogenic mutant of this bacterium and one examining the effect of weekly sprays with fluorescent pseudomonads on the yield of speck-infested field tomatoes, have demonstrated the potential for biocontrol of this pathogen (Cooksey, 1988; Colin and Chafik, 1986). Recently the U.S. Environmental Protection Agency registered the biological control agent *Pseudomonas fluorescens* A506 (also known as BlightBan A506) for use in the control of fire blight on apple and pear and for use in frost protection of

several crops including tomato (Wilson and Lindow, 1993). It is the first MBCA for a foliar bacterial pathogen to be commercialized in the U.S. Because of the tremendous variability that can occur on leaf surfaces with regard to both the physical environment (temperature, water vapor pressure, pH, radiation load, etc.) and the composition and spatial distribution of microbial communities (Upper, 1991), the effectiveness of an MBCA such as A506 may vary from location to location. In 1996, a collaborative study involving Auburn University (Alabama) (M. Wilson), University of Florida (J. Jones), and Agriculture and Agri-Food Canada (D. Cuppels) demonstrated that *Pseudomonas fluorescens* A506 and another biocontrol agent, *Pseudomonas syringae* Cit7, significantly reduced bacterial speck and spot disease severity and incidence on field-grown tomato plants in Ontario and Alabama but not in Florida (Wilson *et al.*, 1996; unpublished data). Because of the complexity of the interactions occurring between potential biocontrol agents and their physical and biological environments, it is essential that we continue to seek new strains that are well-adapted to and can compete at the site where they will be used (Handelsman and Stabb, 1996; Cook *et al.*, 1996).

A second alternative to copper-based pesticides for the control of bacterial diseases of tomato and pepper are microorganisms or chemicals that are able to activate the plant's own defense mechanisms. This inducible response of the plant may or may not involve what is known as systemic acquired resistance (SAR). SAR has been the subject of extensive research in recent years (Chen *et al.*, 1996; Kessmann *et al.*, 1994; Hunt *et al.*, 1996) and has been shown to provide long-term protection in non-infected tissues to a broad spectrum of pathogens. Novartis has developed a chemical activator for use as a foliar spray which is called 'CGA-245704', 'BTH' or 'ActigardTM.' This compound, which is a benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl

ester, activates the plant's defense mechanisms against a wide range of diseases at low use rates. It has no toxicity for mammals, birds, bees and earthworms. Because it has no direct effect on the pathogens (and thus is not considered a bactericide or fungicide), it is unlikely that the pathogen will develop resistance. Another chemical, salicylic acid (SA), has been shown to play an important role in the SAR induction in many plant species. Methyl salicylate (MeSA), a volatile synthesized from SA (and better known commercially as oil of wintergreen), was reported to act as an airborne signal for SAR against tobacco mosaic virus in tobacco (Shulaev et al. 1997). MeSA is assumed to be converted to SA which then induces SAR. If applied as a fumigant rather than a spray, MeSA does not have the phytotoxicity associated with SA sprays, is uniformly distributed to all seedlings and is not displaced by watering or by the application of pesticides to the foliage. Its effect on tomato diseases is unknown.

Development of an effective disease management package based on biocontrol will provide the tomato and pepper industry with a means of reducing chemical pesticide usage, lowering populations of copper-resistant strains in the environment and preventing the buildup of heavy metal residue in soil. Although biocontrol studies currently are in progress in the U.S., their biocontrol agents might not work effectively under Ontario conditions (Cook et al., 1996; Handelsman and Stabb, 1996). Once effective microbial pest control agents are identified, it should be possible to develop an integrated approach combining one or more of the microbial pest control agents with a reduced-frequency copper spray program or with a plant resistance activator such as BTH.

OBJECTIVES

Primary Objective:

To produce an effective biocontrol package for bacterial diseases of tomato and pepper based on microbial antagonism and the induction of disease resistance by natural products.

Specific Objectives:

1. Perform growth room trials to determine the efficacy of selected SAR-inducing microorganisms in controlling bacterial spot, canker and speck.
2. Perform growth room trials to determine the disease control efficacy of applying chemical (methyl salicylate or BTH) SAR inducers to tomato and pepper plug seedlings (to maximize the number of parameters tested in the growth space available, only tomato seedlings were tested).
3. Perform field trials to determine the disease control efficacy of applying chemical and microbial SAR inducers to greenhouse-grown plug seedlings (because none of our microbial strains induced SAR, we ran our trials with promising biocontrol strains from our collection).
4. Compare the efficacy of a disease control package consisting of greenhouse-applied SAR inducers in combination with field-applied microbial antagonists with that of Kocide.
5. Perform growth room trials to determine the effect of this disease control package on selected fungal diseases of tomato.

MATERIALS AND METHODS

Bacterial and fungal strains and growth conditions. The bacterial and fungal strains used in this study are listed in Table 1. Nutrient broth-yeast extract (NBY) was used to grow inocula for the pseudomonad and xanthomonad strains used in this study. The *Bacillus* strains were grown in trypticase soy broth (TSB) or agar (TSA) at 25°C. For the field population studies, *P. syringae* pv. *tomato* was plated on the semiselective Vogel-Bonner-tartrate medium (VBTar)(Cuppels, 1986) and *P. fluorescens* A506 was plated on King's B medium supplemented with 50 µg/ml rifampicin, 75 µg/ml cycloheximide, 10 µg/ml streptomycin. The bacterial spot pathogen was plated on CKTM (Sijam et al., 1991).

Effect of MBCA drenches on bacterial spot of plug seedlings. Tomato seeds were sown in 288-well plug trays containing Promix PGX and grown as described in O.M.A.F.R.A. Factsheet 94-061 (Garton et al., 1994) using a fertilization program recommended by J. Dick. (J. Dick, 1996). The same procedure was used for growing pepper seedlings except that the 288-well plug trays were replaced with the 200-well trays recommended for late season peppers. When the seedlings were 3-weeks-old, the roots of each seedling were drenched with 1 ml of a MBCA suspension (10^8 cfu/ml in sterile water); the drench was repeated in 7 days. Negative controls were drenched with 1 ml sterile water and positive controls were drenched with 1 ml BTH (Actigard 50 WG, a gift from H. Wright, Novartis Crop Protection Canada, Inc.)(62 mg/L water, equivalent to 35 g a.i./ha). Plants were spray-inoculated with the bacterial spot pathogen *X. c.* pv. *vesicatoria* DC96-1 (10^8 cfu/ml in sterile water) 1 to 3 days after the second drench. The treated seedlings were arranged in a completely randomized design with 4 replications (8 plants/rep) for each treatment and each experiment being repeated twice. Disease incidence and severity readings (Fig. 2) were taken 10 to 12 days after inoculation.

Table 1. Bacterial and fungal strains used in this study.

Microbial strain	Disease (host)	Source or reference
Bacterial pathogens		
<i>Xanthomonas campestris</i> pv. <i>vesicatoria</i>		
DC93-1	Bacterial spot (tomato/ pepper)	D. Cuppels (Tomato, Ontario)
DC96-1	"	D. Cuppels (Pepper, Ontario)
<i>Pseudomonas syringae</i> pv. <i>tomato</i>	Bacterial speck	D. Cuppels (Tomato, Ontario)
DC96-3	(tomato)	
Fungal pathogens		
<i>Verticillium dahliae</i>	Verticillium wilt	J. Robb (U. of Guelph)
122 (race 1)	(tomato)	
<i>Phytophthora infestans</i> 1089	Late blight (tomato)	H. Platt (AAFC, PEI)
<i>Fusarium oxysporum</i> f.sp. <i>lycopersici</i>	Fusarium wilt (tomato)	V. Higgins (U. of Toronto)
UT 22 (race1)		
Biocontrol agents		
<i>Pseudomonas syringae</i> Cit7	-	M. Wilson (Citrus, California)
<i>Pseudomonas fluorescens</i> A506	-	Plant Health Technologies, ID
<i>Pseudomonas putida</i> BA-8	-	F. Sahin (Tomato, Ohio)
<i>Burkholderia cepacia</i>		
BA-7	-	F. Sahin (Tomato, Ohio)
BC-161	-	D. Cuppels (Tomato, Ontario)
<i>Micrococcus luteus</i> BC-103	-	D. Cuppels (Tomato, Ontario)
<i>Bacillus</i> (<i>Paenibacillus</i>) sp.		
OSU-140	-	F. Sahin (Tomato, Ohio)
OSU-142	-	F. Sahin (Pepper, Ohio)

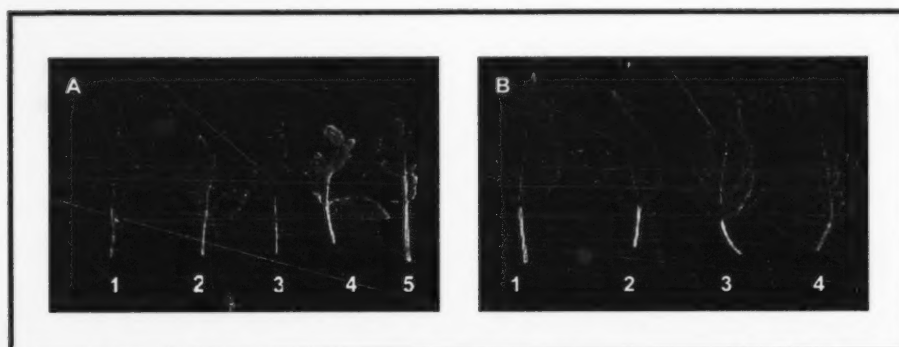


Figure 2. Disease severity rating system for bacterial spot on tomato (A) and pepper (B) plug seedlings. Plug seedlings, grown in 288-well plug trays under standard plug greenhouse conditions, were subjected to various control agents at 21 and 28 days after planting and then, at 30 days, spray- inoculated with *X. campestris* pv. *vesicatoria* DC96-1. Readings were taken 10 days after inoculation. 1, no disease; 2, a few lesions; 3, many spots with some coalescence; 4, severe infection with defoliation; 5, dead plants. No dead pepper seedlings were observed.

Effect of BTH, alone and in combination with MBCAs, on plug seedling growth and bacterial disease severity. Tomato and pepper plug seedlings were grown as described above. When the seedlings were 3-weeks-old, their roots were drenched (1 ml/plug) with various concentrations of BTH; the drench was repeated at 7 days. When treatment consisted of an MBCA, alone or with BTH, the MBCA was applied as a spray (10^7 - 10^8 cfu/ml) at the same time as the BTH drenches. The pathogen was sprayed on the plants as described above; disease readings were taken 10 to 12 days after inoculation. The plants in each eight-plant rep (severed from the roots at soil level) then were dried at 60°C for 48 h and weighed to determine the effect of the various treatments on plant growth.

Effect of MeSA on the pathogen populations of tomato plug seedlings. Tomato (cv. N1045) plug transplants were grown as described above. Four to 5-week old plants were incubated for up to 24 h in enclosed chambers containing a beaker of MeSA in 95% ethanol and a small fan to insure uniform fumigation of the seedlings. Unless otherwise noted, the MeSA concentration routinely used was 250 ug/L of chamber space. Untreated controls were fumigated similarly but with only 95% ethanol in the chamber. For comparison, another series of seedlings was sprayed with a solution of 62 mg/L BTH. One or more weeks after MeSA or BTH treatment, the seedlings were inoculated by intercellular infiltration with a bacterial suspension (10^6 cells/ml). Five days later 9 leaf discs (0.5 cm dia) were collected from inoculated areas of 3 plants for each of 8 replicates and homogenized in 1mM $MgCl_2$ before being plated on CKTM (bacteria spot) or VBTar (bacterial speck). The mean number of colony forming units (cfu)/leaf disc was then determined.

Effect of BTH on fungal diseases of tomato plug seedlings. Tomato (cv. Moneymaker, no known genes for resistance to any fungal diseases) seedlings were grown under the conditions described above. One week before transplanting each plug was drenched with 1 ml BTH (62 mg/L) or water (untreated controls). One week later each seedling was inoculated by dipping "clipped" roots into either water (uninoculated control) or a spore suspension (ca 10^6 to 10^7 conidia/ml) of either *Fusarium oxysporum* f.sp. *lycopersici* or *Verticillium dahliae*. Seedlings were then transplanted into Styrofoam coffee cups containing Metro-Mix and grown at 22 °C on a "plant mobile"(photoperiod 14 h). Plants were sprayed with BTH (62 mg/L) one day after transplanting and the height and number of leaves of all plants were recorded. The same readings were taken at 1 week intervals thereafter for 3 weeks. After the fourth measurement, plants were

clipped at soil level, the fresh weight of each shoot recorded, the vascular system checked for discoloration and cross sections made from 10 plants/treatment for histological examination.

Late blight assays on field grown tomatoes: Tomato plants grown and sprayed with BTH (but not with microbials), as described for the 1999 field trials, were sampled at biweekly intervals by excising one (week 1), or two, mature leaflets per plant for each row (6 or 7 plants for 3 or 4 rows per treatment). BTH was sprayed in the alternate week to sampling. Samples collected on Julian days 167, 181, 194 and 209 thus had received 1, 2, 3, and 4 BTH treatments, respectively. On the days of sampling, excised leaflets were floated abaxial side up on distilled water in Petri plates and inoculated with two 30 µl drops of sporangial suspension of *Phytophthora infestans* P1089 (Vleeshouwers et al., 1999). Leaflets were incubated in the growth chamber (18°C day/16°C night; 12-h photoperiod) for 1 wk. The amount of late blight was assessed after 7 or 9 days by estimating the percentage leaf area diseased. Leaflets were also photographed.

Field study design-1998. Three field plots were established at the London experimental farm of the Southern Crop Protection and Food Research Centre to test the efficacy of BTH and two biocontrol agents at controlling bacterial spot on field tomato (cvs. H9478 and N1045) and pepper (cv. Merlin) plants. There were 7 treatments: BTH (32 mg/L water), MBCA strain A506 (10^8 cfu/ml water), MBCA strain OSU-142 (10^6 to 10^7 cfu/ml water), BTH + A506, BTH + OSU-142, Kocide 101(5 g/L water)-Bravo 500 (9 ml/L water) and the control (water spray, no treatment). Seven-week-old seedlings were transplanted (May 26-27) into non-fumigated flat ground beds spaced 0.46 m apart on 1.2 m centers. The beds were fertilized at planting with 90-190-340 kg/ha. Overhead sprinkler irrigation was applied 3 times in June. A randomized complete

block design with 7 blocks was used for the pepper plot and for each of the two tomato plots. The MBCAs were applied as weekly sprays and the BTH was applied as a spray every 10 days from 2 weeks after planting until 1 to 3 weeks before harvest. Kocide-Bravo was applied weekly starting 3 weeks after planting for tomatoes and 5 weeks after planting for peppers (the pepper plants had taken longer to establish themselves in the field). *X. campestris* pv. *vesicatoria* DC93-1 was applied as a light mist (10^8 CFU/ml) to the tomato guard rows 3 weeks after planting; *X. vesicatoria* DC96-1 (10^8 CFU/ml) was applied to both the treated plants and the guard rows in the pepper plot 38 days after planting. As a control on the effect of these treatments on yield, separate identical plots which were not exposed to bacterial spot were established at another location on the farm.

Field-1999. The same field study design, with 3 experimental plots, was used in 1999. However, instead of 2 tomato cultivars, we used just cv. H9478 and tested for control of both bacterial speck and bacterial spot. The same 7 treatments used in 1998 were applied to the pepper plot and to the tomato-spot plot except that the BTH concentration was increased to 62 mg/L for tomatoes (maintained at 32 mg/L for pepper) and applied every 14 days instead of every 10 days. For the tomato-speck plot, we used the following treatments: BTH (61 mg/L water), MBCA *P. syringae* strain CIT7 (10^8 cfu/ml water), MBCA strain A506 (10^6 to 10^7 cfu/ml water), BTH + A506, BTH + CIT7, Kocide 101(5 g/L water)-Bravo 500 (9 ml/L water) and the control (water spray, no treatment). The plots were irrigated once in June and twice in July. The MBCAs were applied as weekly sprays and the BTH was applied as a spray every 14 days from 2 weeks after planting until 1 to 3 weeks before harvest. Kocide-Bravo was applied weekly starting 2 weeks after planting for

tomatoes and 3 weeks after planting for peppers. The pathogenic strain used for the tomato-speck plot, *P. syringae* pv. *tomato* DC96-3, was applied to the guard rows as described above.

Yield determination and disease assessment for the field plots. Incidence of foliar disease was expressed as the mean number of infected leaves per 50-leaf sample with 4x 50-leaf samples collected per replicate row, or a total of 16x 50-leaf samples per treatment. Severity of foliar disease was expressed as the mean number of lesions/20-leaf sample with 4x 20-leaf samples collected per replicate row (16 samples/treatment). Fruit yield (kg/10 plants) and fruit disease incidence (% infected fruit) were determined 1-2 weeks after the last spray application. Rotting, dark green or very small fruit were not counted. Analysis of variance was performed using the ANOVA or GLM procedures in SAS (SAS Institute, Cary, NC). Means were compared using Duncan's multiple range test at $P=0.05$.

Enumeration of bacterial populations in field experiments. Two 10-g leaf samples were randomly collected from the 40 plants of each treatment and each plot. Each 10-g sample was processed as described previously (Cuppels and Elmhirst, 1999). The bacteria collected from each sample were plated onto CKTM agar to determine the *X. vesicatoria* population, or to determine the *P. syringae* pv. *tomato* population. Colony lifts were made of the appropriate CKTM and VBTar plates and hybridized with the *X. vesicatoria* probe KK1750 (Kuflu and Cuppels, 1997) or the *P. syringae* pv. *tomato* probe TPR1 (Cuppels et al., 1990) by standard procedures (Sambrook et al., 1989). The probes had been DIG-labeled as previously described (Kuflu and Cuppels, 1997).

RESULTS & DISCUSSION

Effect of BTH and MBCAs , alone and in combination with each other, on plug seedling growth and bacterial disease severity. Biocontrol strains from Ohio (*Burkholderia cepacia* BA-7, *Pseudomonas putida* BA-8, *Bacillus* sp. OSU-140 and *Bacillus* sp. OSU-142), and Ontario (*Pseudomonas putida* BC-161, *Micrococcus luteus* BC-103) were antagonistic against a wide range of bacterial and fungal pathogens of tomato based on agar plate assays (Ohio and Ontario data, not shown). These strains, as well as *P. fluorescens* A506 (a biocontrol strain for fire blight of pears and apples; Wilson and Lindow, 1993), were tested for the ability to induce resistance to bacterial spot in tomato (cv. H9478 and cv. N1045) and pepper (cv. Jalapeno and cv. Merlin) seedlings grown according to plug greenhouse specifications. BTH served as a positive control. Although none of these strains induced disease resistance, OSU142 (Ohio), caused a marked increase in seedling growth. BTH, applied as a spray at the rate (62 mg/L) recommended by Novartis, reduced disease severity but also slowed the growth of most cultivars (data available upon request). The deleterious effect of BTH seemed to be cultivar-dependent. Tomato cv. H9474, for example, seemed relatively unaffected. Reducing the concentration of BTH enhanced growth and still protected the seedlings from disease. When tomato plants drenched with BTH were sprayed with OSU142, their growth surpassed that of the controls. Thus, BTH applied at a reduced concentration or in combination with microbials that promote plant growth, such as OSU142, may offer the best alternative to copper-based bactericides for control of bacterial diseases of tomato and pepper plug seedlings.

Effect of MeSA and BTH on bacterial populations of tomato plug seedlings. The injection inoculation method and subsequent estimates of bacterial pathogen populations within inoculated tomato leaflets proved a reliable method to compare the relative degree of disease resistance induced by MeSA and BTH. Both MeSA and BTH reduced *X. campestris* pv. *vesicatoria* populations in the leaf compared to untreated controls (Fig.3). The resistance-inducing effect of MeSA was concentration-dependent up to at least 250 µg/L (Fig. 4a). The resistance induced by MeSA to *X. campestris* pv. *vesicatoria* was detectable one day after treatment and lasted for at least three weeks; the CFU/leaf disc for BTH-treated tomato seedlings averaged 40 to 55% of that recorded for the untreated control plants (data not shown). The resistance-inducing effect of MeSA required exposure to MeSA for 3 to 6 h with an increased effect up to at least 24 h (Fig. 4b). A second MeSA treatment, 1 wk after the first, did not consistently increase the level of protection (Fig. 4c). MeSA fumigation of tomato seedlings also reduced the population of *P. syringae* pv. *tomato* to 66% that of the untreated control (data not shown).

Plants treated with BTH showed reductions in *X. campestris* pv. *vesicatoria* populations equivalent to, or greater than, those seen with MeSA-treated plants. The effect of one BTH application was still evident three weeks later but was quite variable in degree from one experiment to another (the percent reduction in CFU/leaf disc varied from 20 to 50%)(data not shown). When a second BTH application was applied one week after the first one, there was a significant decrease ($P=0.05$) in the bacterial population. Seedlings receiving one BTH spray had populations that were 20 to 21 % of the untreated control while seedlings with two applications had populations that were 8 to 11.4% of the untreated control.

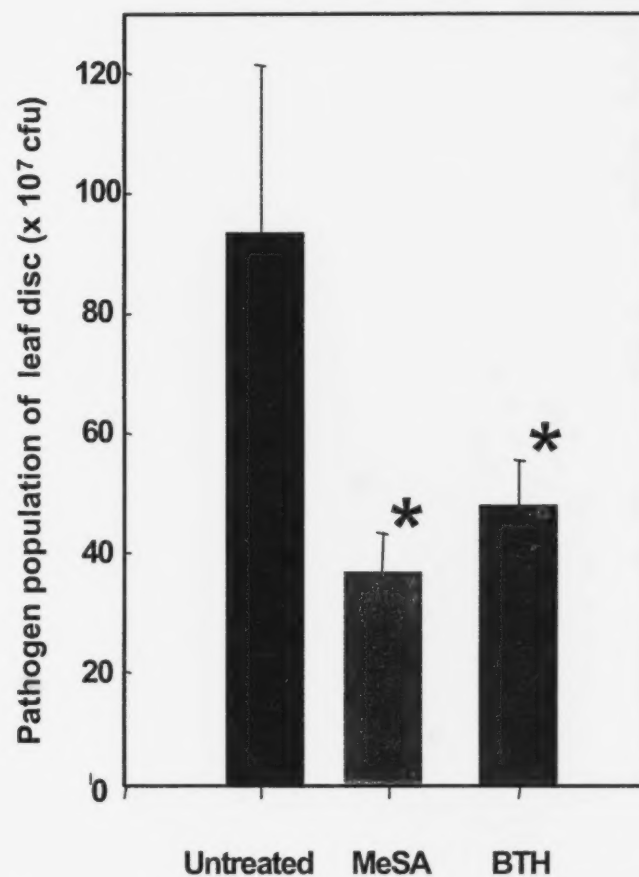


Figure 3. The effect of MeSA (250 μ g/L chamber space) or BTH (62 mg/L) on the *X. campestris* pv. *vesicatoria* population of tomato leaves (cv. N1045). Seedlings were inoculated one week after treatment and the bacterial populations determined five days later. Data are expressed as mean colony forming units/leaf disc. Error bars are standard deviation of the mean (n=8); *, significantly different from the untreated control (P=0.01).

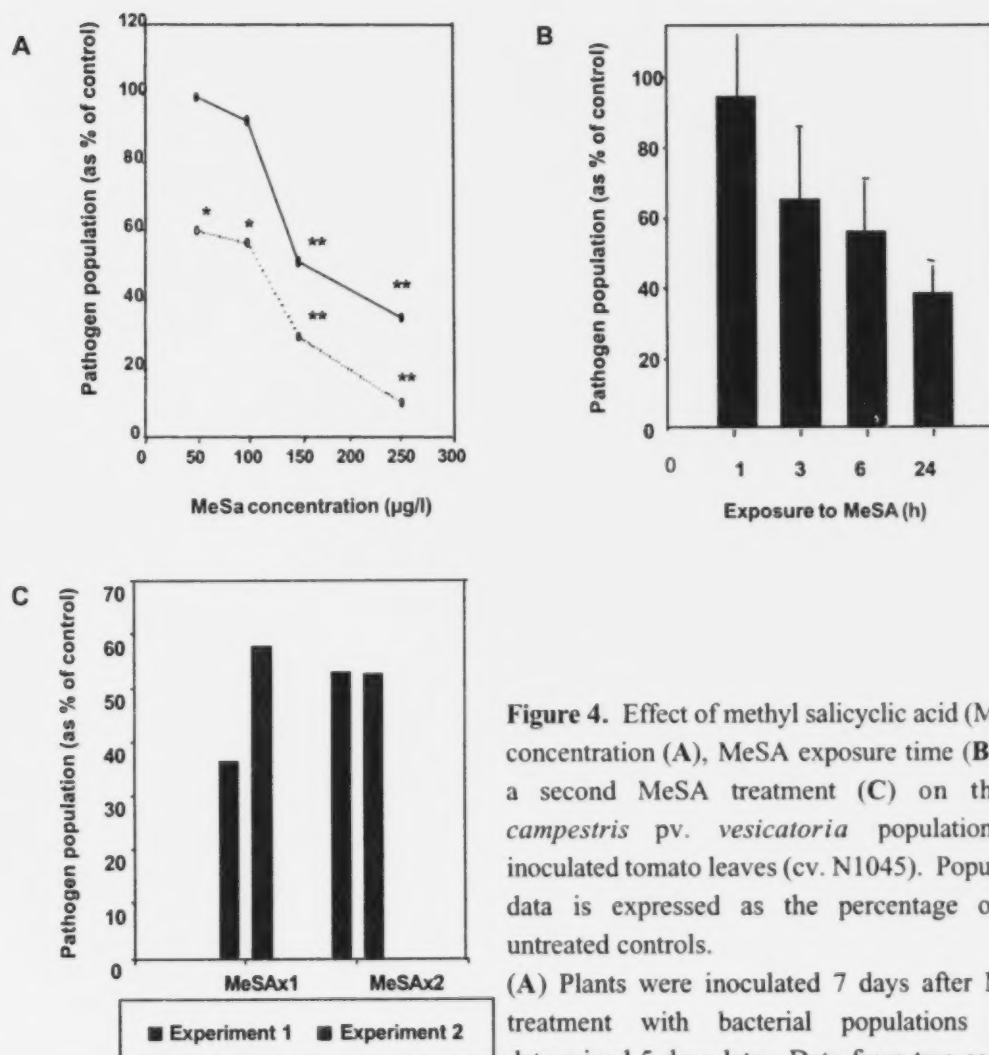


Figure 4. Effect of methyl salicylic acid (MeSA) concentration (A), MeSA exposure time (B), and a second MeSA treatment (C) on the *X. campestris* pv. *vesicatoria* populations of inoculated tomato leaves (cv. N1045). Population data is expressed as the percentage of the untreated controls.

(A) Plants were inoculated 7 days after MeSA treatment with bacterial populations being determined 5 days later. Data from two separate experiments (●-● and ○-○) is shown; **, significant difference at $P=0.01$; *, significant difference from the untreated control at $P=0.05$. (B) Plants were exposed to MeSA (250 µg/L chamber space) for various lengths of time and then inoculated 7 days later. Bacterial populations were determined 5 days after inoculation. Error bars show the standard deviation of the means of two separate experiments. (C) The plants received one (MeSAx1) or two (MeSAx2) MeSA (250 µg/L chamber space) treatments one week apart; they were inoculated one week after the second treatment. Results shown are for two separate experiments; both the MeSAx1 and MeSAx2 treatments were significantly different from the untreated control at $P=0.05$.

Effect of BTH on fungal diseases of tomato plug seedlings. In two separate experiments, BTH appeared to slightly reduce the adverse effect of *Fusarium* on tomato seedling growth (Fig. 5). This pathogen killed several of the inoculated control plants and plants receiving one application of BTH; none of the plants receiving two applications of BTH died. When the treatments were compared using fresh weight measurements, comparable differences were observed (data not shown). There was considerable variability in the experiments testing the effect of BTH on *Verticillium* wilt and no conclusions can be drawn. Additional experiments with *Verticillium* are in progress. All *Verticillium*- and *Fusarium*-inoculated plants had typical vascular browning; the presence of the pathogens was confirmed by isolating them from the vascular tissue of the infected plants. BTH did not have an adverse effect on the cultivar (cv. Moneymaker) used in these experiments.

Field trials of BTH and MBCAs-1998. Because environmental conditions in London were especially favorable for bacterial spot development in 1998 (heavy rains in early July, Fig. 6), the plants in the test plot exposed to the bacterial spot pathogen were severely diseased. For cv. N1045, Kocide and the BTH-OSU142 combination were the only treatments resulting in a significant reduction in disease severity; for cv. H9478, which was not as severely infected, all treatments reduced disease incidence (data not shown) and severity with no significant difference between the treatments (data available upon request). Kocide was the only treatment that significantly reduced disease incidence (by 14.4%) on the fruit but only for cv. H9478. Although the increases were not statistically significant, all four treatments enhanced yield (14-25%) of cv. 9478 but not cv. N1045 (Table 2).

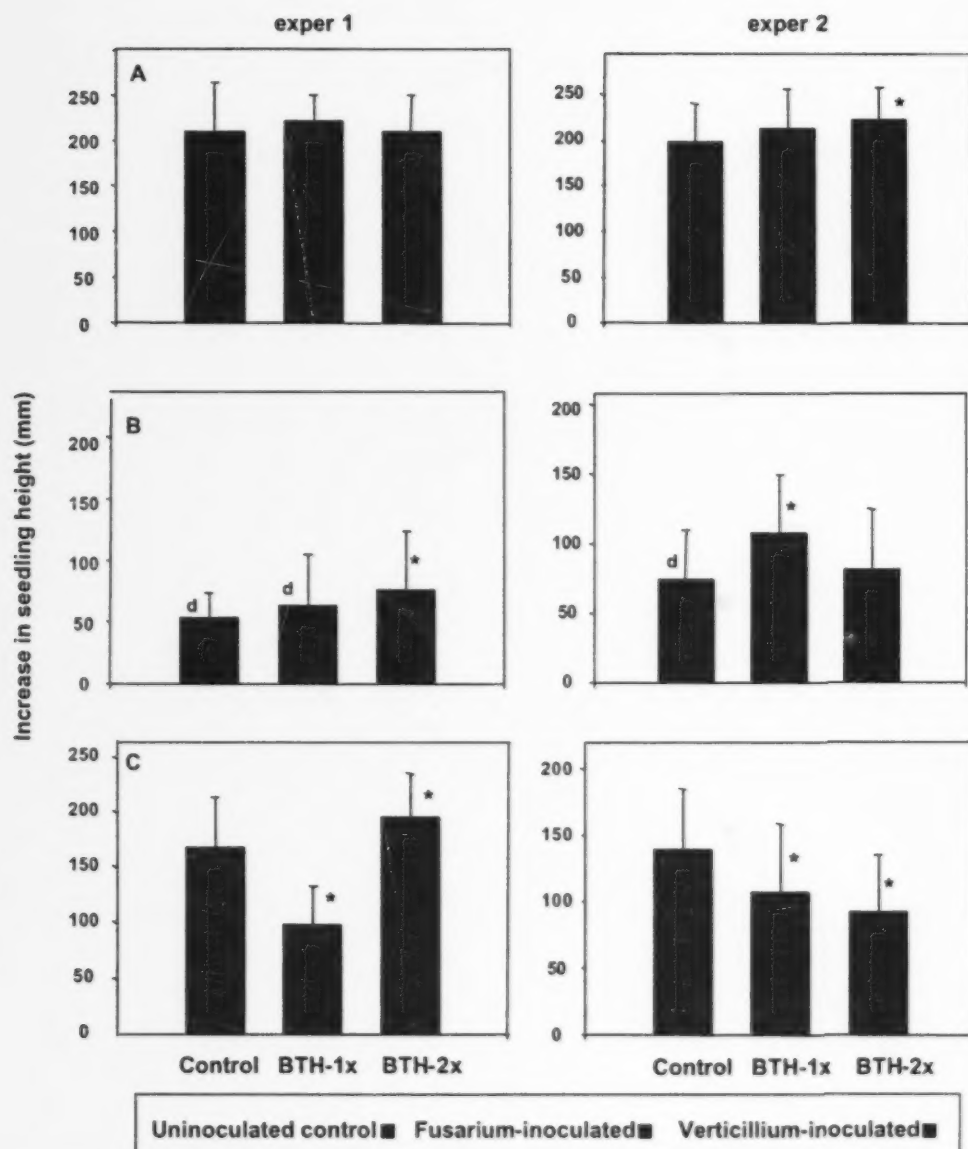


Figure 5. Effect of one (BTH-1x) or two (BTH-2x) BTH treatments on the growth of tomato seedlings (cv. Moneymaker) 4 weeks following no inoculation (A), inoculation with *Fusarium oxysporum* f.sp. *lycopersici* (B) or inoculation with *Verticillium dahliae* (C). The experiment was repeated twice (exper 1 and 2). *, significantly different from the untreated control at $P=0.05$, $n=\text{ca } 35$ for exper 1 and $\text{ca } 20$ for exper 2; d, 3 each of the BTH-1x and control plants died in exper 1 while one of the control plants in exper 2 died (these plants were not included in the mean).

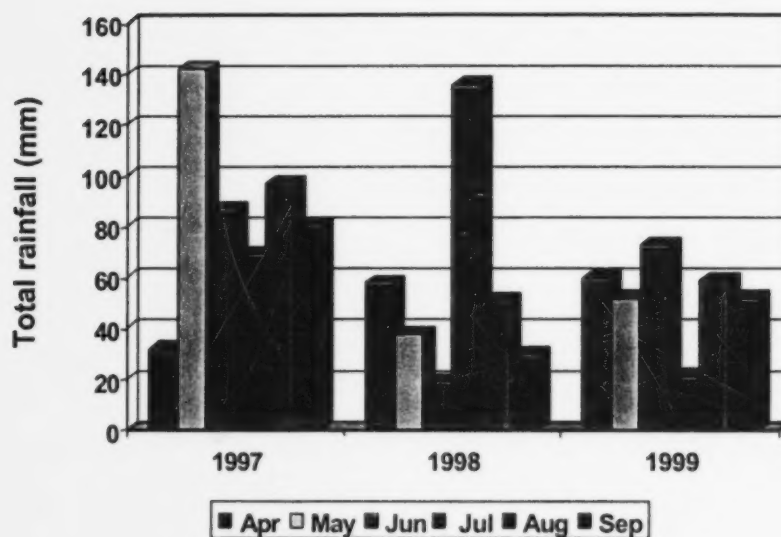


Figure 6. Monthly rainfall on the AAFC-London experimental farm from April to September of 1997, 1998 and 1999. Courtesy of G. McFadden, farm manager, AAFC-London

BTH (37%), BTH-A506 (55%) and BTH-OSU142 (53%) significantly reduced disease severity on pepper (cv. Merlin); Kocide and A506 alone had little effect on this disease. The lack of control with Kocide may have been due to the late date for the first application of this bactericide (5 weeks after planting, due to extremely dry weather and the stressed state of the plug seedlings). In a similar experiment during 1997, A506, BTH and BTH-A506 reduced disease severity by 0%, 33% and 70%, respectively. In the test plot not inoculated with bacterial spot, there were no significant differences in yield for any of the treatments (data available upon request). Some early blight was observed in this plot but it was slight and was effectively suppressed with Bravo 500F (4 L/ha). For cv. N1045 there was a 12-21% reduction in yield between the diseased and healthy plot; for cv. H9478, there was a 34-47% reduction in yield; for cv. Merlin (pepper) there was a 0 to 26% reduction in yield.

Although no significant yield differences between the various treatments was observed in inoculated plots, there was a significant decrease in yield in the BTH and BTH+OSU142-treated peppers in the uninoculated plot. In 1997, when BTH was applied at twice the dosage used in 1998, pepper yield decreased by more than $\frac{1}{2}$ (data not shown). Our field results corroborate our growth room results with regard to the effect of BTH on pepper plant growth; field tomato plants appeared more tolerant of the resistance activator. The population dynamics for *X. campestris* pv. *vesicatoria* in these test plants was also determined (data available upon request). The number of bacteria on the severely diseased tomato plants was extremely high, exceeding 10^8 cfu/g of tissue. Kocide had a significant effect on the populations in all 3 test plots but was not able to reduce disease severity in the pepper plot. Disease severity readings were taken shortly after the last population sampling (Julian day 229), at which time Kocide had little effect on the pepper

pathogen. The pepper pathogen was the only one whose populations were suppressed by BTH. These results differ significantly from those obtained in our plant growth room studies (Fig. 3) where the pathogen population of BTH-treated tomato seedlings was $\frac{1}{2}$ that of the controls. As has been shown with microbial biological control agents, control under greenhouse/plant growth room conditions may not translate into control under field conditions (Cook, 1993).

Field trials of BTH and MBCAs-1999. The MBCAs and bacterial diseases included in the 1999 field trials were selected based on the results of the 1996, 1997 and 1998 field trials (data from 1996 and 1997 not shown). For the tomato-speck plot, the BTH-Cit7 combination gave the best control, reducing disease incidence by 31% and disease severity by 52%; for the tomato-spot plot, the BTH-A506 combination was the only treatment, except for Kocide, that significantly reduced disease severity (36%)(data available upon request). Kocide significantly reduced disease in all three plots (tomato-speck, 40%; tomato-spot, 38%; pepper-spot, 39%). As in previous years (1998-55% and 1997-70%), the best control in the pepper-spot plot was achieved with the BTH-A506 treatment (61%). In the 1998 pepper trial, strain OSU142 seemed to enhance, although not significantly, the control achieved with BTH; in 1999, addition of this bacterium had little effect on BTH control. In fact, OSU142, which was an effective control agent in plant growth room studies, had little effect on disease development in the field. The dry, hot conditions characterizing the 1999 and 1998 (with the exception of 2 heavy downpours in July) growing seasons (Fig. 6) may have affected the ability of OSU142 and that of the other microbial biocontrol agents to multiply on the foliage. Microbes differ substantially in their ability to withstand heat and dryness (Cook, 1993). Being a spore former, strain OSU142 may have entered a dormant state. Strain

A506 was monitored during the 1997, 1998 and 1999 growing seasons (data not shown) and its growth was significantly lower in 1999, particularly during the early part of the season. Bacterial spot is considered a high temperature (optimum at $>24^{\circ}\text{C}$) disease and thus these conditions may not have inhibited on *X. campestris* pv. *vesicatoria* to the same extent (Stall, 1995). In the tomato-spot plot, the only treatment resulting in a significant increase in yield (22%) was Kocide, duplicating the results obtained in 1998; in the tomato-speck plot, there was a 20% increase in yield for the BTH-Cit7 treatment. The exceptionally low pepper yields (2.5 to 4.5 kg/10 plants) obtained in 1999 were probably due to the dry weather; none of the treatments significantly increased yield. Yield data was not collected from our uninoculated test plots (where the tomato and pepper plants received treatments but were not exposed to bacterial speck or spot) in 1999 due to the damage inflicted by insects, early blight and a bacterial canker outbreak. The population dynamics of the two pathogens during the 1999 growing season was determined for the following treatments: control, Kocide, A506, BTH and BTH-A506(data available upon request) . Overall, the populations were 1/100 of their 1998 size, which, most likely, was due to the dry weather. BTH treatments had a marked effect on populations of the bacterial speck pathogen and the bacterial spot-pepper pathogen early in the season; the effect on the bacterial spot-tomato pathogen was not as pronounced but seemed to last longer into the season. Although Kocide reduced disease severity by 38-40%, it had little effect, overall, on pathogen populations in the bacterial speck-tomato plot and the bacterial spot-pepper plot. The bacterial speck disease severity readings were taken on Julian day 200 while the bacterial spot-pepper readings were taken on Julian day 234; at these times Kocide appeared to be suppressing the pathogen populations, which would account for the drop in disease severity that we observed. The bacterial speck strain used in this study was

copper-tolerant, as are virtually all Ontario bacterial speck strains isolated in recent years (Cuppels and Elmhirst, 1999). The bacterial spot-pepper strain used in our study was copper-sensitive but isolates of the pathogen collected from the test plot late in the season were copper-tolerant, indicating that weekly sprays may be promoting copper-tolerance among the bacterial populations growing on foliage.

Effect of BTH on the late blight pathogen *P. infestans*. Although preliminary, detached leaf assays (Table 2, Fig. 7) indicated that BTH caused a significant reduction in lesion size (expressed as percent of leaf area diseased) following the first and second sprays (2 and 4 weeks after planting). The effect was not as pronounced after the third and fourth sprays (6 and 8 weeks after planting) but this result may be an indication that the amount of BTH applied to the plants was inadequate for the size of plants.

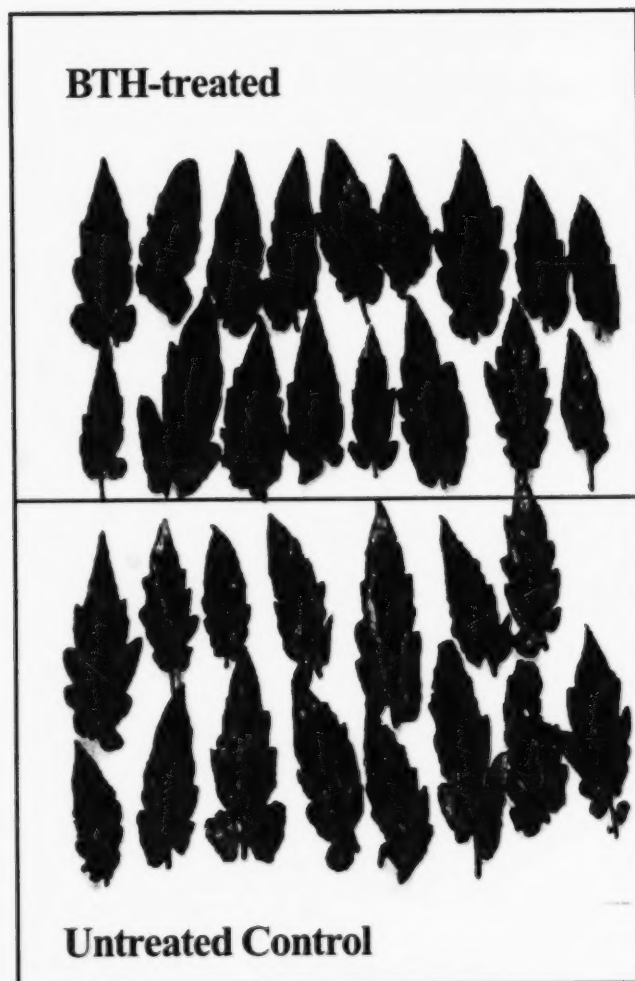


Figure 7. Effect of BTH on the susceptibility of detached leaflets from field grown tomato plants to the late blight fungus *Phytophthora infestans*. The leaves, collected on Julian day 181 (see Table 2), were photographed nine days after inoculation

Table 2. Effect of BTH on the susceptibility of field grown tomato plants to *P. infestans* as measured on excised leaflets.

Sample date ¹ treatment	Infected leaflets (infected/total)	% leaf area infected	SD	95% confidence	Area as % of control
Julian day 167					
C ²	19/19	83.6	15.3	7.4	
BTH-1x ³	15/16	40.3	24.3	12.9	48.2* ⁴
Julian Day 181					
C	17/18	40.3	20.5	10.2	
BTH-2x	36/44	10.8	19.3	9.1	26.8* ⁴
Julian Day 194					
C	43/44	29.5	24.1	7.3	
BTH-3x	36/43	21.2	13.4	4	71.9
Julian Day 209					
C	42/44	32.5	18.2	5.6	
BTH-4x	45/46	24.6	21.4	6.5	75.7

¹ Leaflets (1-2) collected from the field were floated on water, inoculated with *P. infestans* P1089 and incubated for 7 days (9 days for Julian Day 194 samples) in the growth chamber. The amount of late blight was assessed by counting the number of leaflets with symptoms and determining the percentage of leaf area showing symptoms.

² C = Inoculated plants that have not been sprayed with BTH.

³ BTH was sprayed on the field plants at 14-day intervals beginning Julian day 162; 1x = 1 spray; 2x = 2 sprays; etc.

⁴ Significantly different from the inoculated untreated control at P=0.01.

CONCLUSIONS

- None of the biocontrol strains from the Ohio and Ontario collection were able to induce disease resistance in tomato (cv. H9478 and N1045) or pepper (cv. Merlin and Jalapeno) plug seedlings.
- The plant resistance activator benzothiadiazole (BTH) reduced disease severity on plug seedlings (cv. CC318, CC337, N1045, H9478, H9474, Elisa, Merlin and Jalapeno) but had a cultivar-dependent deleterious effect on seedling growth.
- One of the biocontrol strains, *Bacillus* sp. OSU142, had plant growth promoting ability and was able to counteract the deleterious effect of BTH on plug seedlings.
- The plant resistance activators BTH and methyl salicylic acid (MeSA) significantly suppressed *X. campestris* pv. *vesicatoria* populations on plug seedlings; the effect was still evident 3 weeks after treatment.
- The protective effect of MeSA, applied as a fumigant, was concentration-dependent, required a minimum 3 to 6-h exposure time, and did not improve substantially with a second treatment. However, a second BTH treatment significantly enhanced its effect on *X. campestris* pv. *vesicatoria* populations.
- BTH appeared to have little effect on the fungal pathogens *Verticillium dahliae* and *Fusarium oxysporum* f.sp. *lycopersici* although it did arrest late blight (*Phytophthora infestans*) development in a detached leaf assay using treated field tomato leaves.
- In the tomato field trials, BTH was most effective against bacterial speck and spot at a concentration of 62 mg/L applied every 10 or 14 days starting 2 weeks after planting (no reductions in yield were observed); in the pepper field trials, the best control of spot without

yield reduction occurred at a concentration of 32 mg/L applied every 10-14 days starting 2 weeks after planting.

- In field trials over a 3-year period, the amount of disease control achieved with the various microbial biocontrol agents depended upon environmental conditions with the reduction in disease severity rarely exceeding 35%.
- In the field trials, the best control of bacterial spot and speck occurred when BTH was used in combination with a biocontrol agent; the best combinations were BTH plus *P. syringae* Cit7 for bacterial speck and BTH plus *P. fluorescens* A506 for bacterial spot of pepper.
- In 3 (1997-1999) separate field trials, BTH-A506 reduced bacterial spot severity on pepper by 70, 53 and 61%; the best control achieved with Kocide-Bravo was 39%.

FUTURE DIRECTIONS

- Our work has shown that the plant resistance activator benzothiadiazole (BTH) in combination with *Bacillus* sp. strain OSU142 effectively controls bacterial spot on tomato and pepper plug seedlings with no adverse effect on plant growth. The effect of this control treatment on the diseases bacterial speck and bacterial canker should also be assessed.
- A control treatment consisting of BTH (at 31 mg/L applied every 2 weeks) and *P. fluorescens* A506 reduced bacterial spot on field peppers by 60%. This combination was not as effective on field tomatoes infected with spot or speck. Other biocontrol strains do not have this effect when combined with BTH. Future research will be directed at determining how A506 is enhancing BTH-mediated control of bacterial spot on peppers. The information we obtain should help us to design an effective control treatment combination for bacterial spot and speck on tomatoes.
- Preliminary work suggests that *P. syringae* Cit7 may have an additive effect on bacterial speck control by BTH. Additional field testing will be required to confirm this observation.
- Additional testing is needed to confirm the reduction of late blight disease severity by BTH.
- Fumigation with methyl salicylic acid (MeSA) suppressed bacterial spot on tomato plug seedlings. Additional experiments should be performed to determine if overnight fumigation with MeSA would be an effective way of preventing the spread of bacterial and fungal diseases in plug greenhouses.

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GLOSSARY OF TERMS

- BTH** Acibenzolar-S-methyl, Actigard 50WG, CGA 245704 (Syngenta, Basel, Switzerland).
- CKTM** Semi-selective medium for growth of *X. campestris* pv. *vesicatoria*. (Sijam et al., 1991).
- DIG.** Digoxigenin (Roche Molecular Biochemicals).
- MBCA.** Microbial biocontrol agent.
- MeSA** Methyl salicylate.
- NBY** Nutrient Broth Yeast Extract medium.
- SA.** Salicylic acid
- SAR** Systemic Acquired Resistance
- SD.** Standard Deviation
- TSA or TSB.** Trypticase Soy Agar or Broth.
- VBTar.** Vogel Bonner Tartrate medium. Semi-selective medium for the growth of *P. syringae* pv. *tomato*.

